中国病理生理杂志 2007 23 (12): 2460-2464 ISSN: 1000-4718 CN: 44-1187/R

论著

转JAK2基因脐血CD34$^+$细胞体外扩增与生物学特性研究

赵声明,顾惜春,常乃柏△,许晓东,裴蕾

卫生部北京医院血液科,北京 100730

收稿日期 2006-6-13 修回日期 2006-10-16 网络版发布日期 2008-8-29 接受日期 2006-10-16

摘要 目的: 探讨转基因JAK2介导的脐血干祖细胞长期扩增调控的可行性和转基因细胞的生物学特征。

方法: 构建逆转录病毒载体MGI-F_2JAK2，内含有JAK2基因的功能催化区和2个与小分子靶向基因合成药物(AP20187)结合的位点蛋白(F36v，F_2)。应用MiniMACS磁珠分选系统纯化分离脐血CD34$^+$细胞，用含JAK2的逆转录病毒上清转染脐血CD34$^+$细胞。转染后的CD34$^+$细胞在IMDM培养体系中，将细胞分为AP20187组;FL组；TPO组；AP20187+FL+TPO (AFT) 组。对扩增后的细胞定期检测基因转移后GFP动态变化、细胞免疫标记、造血祖细胞集落培养、染色体核型分析和裸鼠致瘤实验。

结果: 分选的CD34$^+$细胞纯度>91%，基因转移率为49.32%±6.21%；只有AP20187+FL+TPO组可以使转基因的脐血CD34$^+$细胞大量增殖，扩增至第8周时细胞数达10^9，CD34$^+$细胞GFP的阳性率由基线水平逐渐上升并于第8周时达到90%以上；细胞表型为CD33$^+$、CD61$^+$、Gly-A$^+$部分阳性；CD38$^+$、HLA-DR$^+$强阳性；CD2、CD7、CD19接近阴性。扩增的CD34+细胞可分别形成BFU-E、CFU-GM、CFU-Mix并以CFU-GM集落为主。扩增后CD34$^+$细胞检测染色体核型正常，裸鼠实验无致瘤特性。

结论: 转染JAK2 基因的人脐血CD34$^+$细胞协同FL和TPO细胞因子可以体外长期扩增脐血干祖细胞，对今后研究细胞信号转导、造血调控以及开展干细胞和基因治疗都有潜在的应用价值。

关键词 酪氨酸激酶JAK2 基因疗法；CD34+细胞 扩增

分类号 R363

Biological characteristics of JAK2 transduced CD34$^+$ cells from cord blood during ex vivo expansion

ZHAO Sheng-ming,GU Xi-chun,CHANG Nai-bai,XU Xiao-dong,PEI Lei

Department of Hematology,Beijing Hospital,Beijing 100730,China.E-mail: mgz2001@hotmail.com

Abstract

<FONT face=Verdana>AIM: To explore the feasibility and biological characterization of long-term regulated expansion of JAK2 transduced human CD34⁺ cord blood cells in vitro.
METHODS: A retrovirus (RV) vector which contains JAK2 catalytic domain and two binding sites for a chemical inducer,dimerization (AP20187),was cloned (designated MGI-F₂JAK2).CD34⁺cells were enriched from cord blood with a MiniMACS system.The purified CD34⁺cells were transfected with supernatant from the retrovirus packaging cell line that expressed JAK2.Following transduction,cells were expanded into four groups: AP20187 alone,FL alone,TPO,alone,AP20187+FL+TPO,respectively.The expanded cells were monitored by GFP expression,immunophenotyping,progenitor colony assay,karyotype analysis as well as tumorigenesis in nude mice.
RESULTS: The purity of selected CD34⁺ cells was over 91% and gene transfer rate was 49.32%±6.21%.Only the group of AP20187 +FL+ TPO was obtained a significant sustained outgrowth of the transduced CD34⁺ cord blood cells.The percentage of GFP+ cells consistently produced a rise to the 90% peak level by the end of 8th week of culture.Flow cytometry analysis showed that the phenotype of the expanded cells was CD33⁺,CD61⁺ and Gly-A⁺ partial positive;CD38⁺ and HLA-DR⁺ strong positive,while CD2,CD7 and CD19 were almost negative.Colony assays performed in methycelluos,which can give rise to BFU-E,CFU-GM and CFU-Mix,the CFU-GM was predominantly in all colonies.The tumor

was not observed in nude mice and the karyotype analysis was normal from expanded cells.
CONCLUSION: The results demonstrate that AP20187-mediated activation of JAK2 signaling is capable of stimulating expansion JAK2 transduced CB CD34⁺ cells in combination with FL and TPO.This system may have applications for studies in signaling transduction,hematopoiesis,and for gene and cell therapy.</FONT>

Key words Tyrosine kinase JAK2 Gene therapy CD34+ cells Expansion

DOI: 1000-4718

通讯作者 常乃柏 mgz2001@hotmail.com